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Direct Evidence for Granuloma-Inducing Activity of Interleukin-1

Induction of Experimental Pulmonary Granuloma Formation in Mice by Interleukin-1-Coupled Beads

KEITA KASAHARA, MD,
KAZUO KOBAYASHI, MD,
YUSUKE SHIKAMA, MD,
IKUYO YONEYA, MD,
KAZUHIKO SOEZIMA, MD,
HIROTSUGU IDE, MD, and
TERUMI TAKAHASHI, MD

From the First Department of Internal Medicine, and Pathology, School of Medicine, Showa University, Tokyo, Japan

Pulmonary granulomas were induced in BALB/c mice by the intratracheal injection of Sephadex G-50 and latex beads. Very large granulomas developed around Sephadex G-50 beads. Minimal inflammation was produced in mice given latex beads. Aqueous extracts prepared from pulmonary granuloma lesions induced in mice by Sephadex G-50 beads contained high levels of interleukin-1 (IL-1) activity but not interleukin-2 (IL-2) activity. IL-1 activity in the extracts correlated with granuloma size. In a subsequent step, large granulomas were induced by the intratracheal injection of Sepharose 4B beads coupled to fractions of the extracts containing IL-1 activity (ie, granuloma-derived IL-1) prepared from Sephadex G-50-induced granulomatous lungs. In addition, large granulomas were induced by the intratracheal injection of recombinant IL-1-coated Sepharose 4B beads. In contrast, very small granulomas were seen when IL-2-coated or plain Sepharose 4B beads were injected into mice. These results indicate that IL-1 participates in the induction and/or expression of granulomas. (Am J Pathol 1988, 130:629–638)

GRANULOMAS are focal, predominantly mononuclear tissue inflammations evoked by persistent irritants. The composition of the irritant and its degradability and immunogenicity are decisive factors in the etiology (nonimmune, foreign-body, or hypersensitivity) and development of the lesion.[1] Granulomatous inflammation is associated with many significant human diseases, including tuberculosis, sarcoidosis, leprosy, schistosomiasis, and berylliosis. Very little is known about the fundamental mechanism of this type of inflammation. Histopathologically, the bulk of both hyersensitive and foreign-body granulomas is composed of macrophages and their derivatives.[2] Studies have shown that cytokines such as interleukin-1 (IL-1), migration inhibition factor, and chemotactic factors play a role in hypersensitivity granuloma formation,[3–7] although direct evidence suggesting the ability of cytokines to induce the lesion is still lacking. The mechanism of foreign-body granuloma formation remains unknown.

In order to analyze the basic mechanism of granuloma formation, we induced granulomas in mice by injecting Sephadex G-50.[8] A direct correlation was demonstrated between the intensity of Sephadex G-50-induced pulmonary granulomas and IL-1 activity of the lung extracts in mice. In addition, the results

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Address reprint requests to Kazuo Kobayashi, MD, First Department of Internal Medicine, School of Medicine, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan.

demonstrated in this paper strongly suggest that IL-1, but not interleukin-2 (IL-2), is involved in the induction and/or development of granulomas in mice.

Materials and Methods

Mice

Female BALB/c mice were purchased from Charles River Japan, Tokyo. All mice were used at 6–8 weeks of age.

Induction of Pulmonary Granulomas by Sephadex G-50 Beads

Pulmonary granuloma formation was induced according to a method previously described.[8] Briefly, mice were injected intratracheally with a 0.1-ml suspension of Sephadex G-50 (Pharmacia Fine Chemicals, Uppsala, Sweden) or latex (Polysciences Inc., Warrington, Pa) at 1.5×10^5 beads/ml. These beads were sterilized by autoclaving before use.

Histologic Examination

Histologic sections were made from each excised lung. These were prepared at a thickness of 5 μ and were stained by hematoxylin and eosin (H&E). According to a described method,[6,8,9] the intensity of granulomatous reactions was quantitated by measuring the radius of inflammation surrounding the injected beads. Beads 50–80 μ in diameter were selected for measurement. At least 10 granulomas from different sections of each lung were measured and were reported as the mean radius (μ) ± SEM. We performed three to four independent experiments of three mice per each condition. Thus, the mean ± SEM was derived from the determinations of 90–120 granulomas. Under this condition, SEM averaged less than 5–10%.

Preparation of Lung Extracts

Aqueous extracts of granulomatous lungs were prepared by methods previously described.[6,8,9] Briefly, lungs were inflated with 1 ml physiologic saline and were homogenized in 5 ml of saline by using a Polytron (Brinkmann Instruments, Westbury, NY) for 30 seconds. Tissue was kept on ice during these procedures. Homogenized tissues were then centrifuged in a refrigerated unit at 2000*g* for 30 minutes, and the tissue pellet was discarded. Samples were then sterilized with a Millipore membrane (pore size, 0.45 μ) and were stored at −70 C until use. The lung extracts contained 0.4–0.5 mg protein/ml saline as described previously.[6,8,9]

Gel Filtration Chromatography

Gel filtration chromatography was performed at 4 C on a Sephacryl S-200 (Pharmacia Fine Chemicals, Uppsala, Sweden) column (85 × 2.2 cm, Amicon, Lexington, Mass). The column was equilibrated with phosphate-buffered saline (PBS) and calibrated with molecular weight standards (Pharmacia Fine Chemicals): blue dextran (BD; exclusion marker), human IgG (150,000 mol wt), bovine serum albumin (BSA; 67,000 mol wt), chymotrypsinogen A (CTN; 25,000 mol wt), and cytochrome C (CC; 12,000 mol wt). Fractions of 4 ml were collected and were lyophilized and reconstituted to the fivefold concentrated volume of the original sample. The fractionated samples were coupled to cyanogen bromide-activated Sepharose 4B beads (Pharmacia Fine Chemicals), and residual active groups of both protein-coupled and -uncoupled beads were blocked by 0.5 M Tris-HCl buffer (Sigma Chemical Co., St Louis, Mo) as described.[6,9] Minimal pulmonary reaction was seen when the beads blocked by adding of BSA, ethanolamine, or Tris-HCl were injected into mice; therefore, we used Tris-HCl for blocking the remaining active group. Approximately 70–80% of proteins were bound to the beads. The protein concentration was examined by using an ultraviolet spectrophotometer at 280 nm. Granuloma-inducing activity was examined by the intratracheal injection of fractionated sample-coupled beads into mice (1.5×10^4 beads/0.1 ml/mouse). Beads 50–80 μ in diameter were selected for measurement. The fractionated samples were also assayed for cytokine activities.

Cytokines

Recombinant human IL-1β with a specific activity of 2×10^7 U/mg was kindly provided by Dr. Y. Hirai, Immunological Products and Development, Otsuka Pharmaceutical Co., Tokushima, Japan.[10] Recombinant human IL-2 with a specific activity of 1×10^7 U/mg was a generous gift from Shionogi Pharmaceutical Co., Osaka, Japan.[11] Recombinant cytokines were coupled to Sepharose 4B beads (Pharmacia Fine Chemicals), and the residual active groups were blocked. Approximately 70–80% of cytokine activities were bound to the beads examined by bioassays described below. Thus, $1–2 \times 10^3$ U of cytokine activity (IL-1 or IL-2) was bound to Sepharose 4B beads at 1.5×10^4 beads. Mice were injected intratracheally

with a 0.1-ml suspension of cytokine-coupled or -uncoupled Sepharose 4B beads at 1.5×10^5 beads/ml.

Bioassays for Cytokine Activities

The thymocyte proliferation activity of IL-1 was determined by its capacity to stimulate BALB/c mouse thymocytes in the presence of phytohemagglutinin (PHA; Difco Laboratories, Detroit, Mich), as described.[12] IL-2 activity was assayed by its ability to stimulate proliferation of the IL-2-dependent cell line CTLL-2.[13] The CTLL-2 was kindly provided by Dr. K. A. Smith. In all assays, a unit of activity was defined as the amount of materials per milliliter producing a half maximal response.

Data Analysis

Statistical analysis of data with respect to controls was performed with the Student t test; P values less than 0.05 were considered significant.

Results

Lung Granuloma Formation by the Intratracheal Injection of Sephadex G-50

In mice given intratracheal injections of Sephadex G-50 beads large foreign-body granulomas developed that were conspicuous by Day 1, reached maximum size (40–50-μ radius) by Day 3, and then gradually decreased in size thereafter. A characteristic lesion is shown in Figure 1A. The lesions were composed predominantly of macrophages with fewer neutrophils. The inflammatory reactions induced by latex beads in mice were considerably smaller and were composed of macrophages (Figure 1B). A summary of the time course of granuloma formation is shown in Figure 2.

IL-1 Activity in Lung Extracts

As shown in Figure 3, IL-1 activity in lung granuloma extracts was detectable within 1 day and reached peak activity by 3 days after the intractracheal injection of Sephadex G-50 beads into mice. A rapid decrease in IL-1 activity was observed 7–14 days after injection. However, no IL-1 activity was observed in lung extracts prepared from normal mice or mice given latex beads. The temporal profile of IL-1 activity in the extracts was almost identical to the profile of granuloma development (Figure 2). In addition, both Sephadex G-50 and latex beads themselves appeared to have no direct effect on this assay, because extracts prepared from mice given either Sephadex G-50 or latex beads prior to sacrifice (Day 0) showed no IL-1 activity. Because thymocyte proliferation assay with a suboptimal concentration of PHA would reflect both interleukin (IL-1 and IL-2) activities in the samples,[14,15] we therefore examined IL-2 activity in lung extracts using IL-2-dependent CTLL cells in a proliferative assay in order to clarify the nature of thymocyte proliferative response in the standard IL-1 assay described above. No IL-2 activity was detected in the lung extracts that had thymocyte proliferative activity (data not shown).

Lung Granuloma Formation Induced by the Intratracheal Injection of Sepharose 4B Beads Coupled With IL-1-Containing Fractions (ie, Granuloma-Derived IL-1) Obtained From the Lung Granuloma Extracts

To explore the role of cytokines in granuloma formation, we first attempted to produce passive granuloma formation by using granuloma extracts. Lung extracts prepared from granuloma-bearing mice (Day 3) induced by Sephadex G-50 were applied to Sephacryl S-200 gel filtration chromatography. As shown in Figure 4, chromatography of lung extracts prepared from granuloma-bearing mice on Sephacryl S-200 revealed the presence of at least two major peaks of IL-1 activity with molecular weight in the range of 12,000–25,000 and 25,000–67,000. As described above, no detactable IL-2 activity was found in the extracts as well as fractionated samples. Next, each fraction was coupled to Sepharose 4B and then injected into mice. Granuloma formation was induced in mice upon injection of Sepharose 4B beads coupled to either IL-1-containing fraction (Figure 4). The resultants infiltrates were primarily composed of macrophages and scattered neutrophils (Figure 5A). The histologic features and time-kinetics of the granulomas were similar to those induced by Sephadex G-50 (Figure 1A). In contrast, very small lesions were observed in mice given Sepharose 4B coupled to fractions that did not contain IL-1 (Figure 5B). Additionally, minimal pulmonary reaction was seen in the mice given beads coupled to fractionated extracts prepared from normal mice and mice given uncoupled beads. Thus, IL-1 activity correlated well with granuloma-inducing activity in the extracts.

Granuloma Formation Induced by Injection of Recombinant IL-1-Coupled Sepharose 4B Beads

Based on the results that IL-1 activity correlated well with granuloma-inducing activity, we then examined the ability of pure IL-1 to induce granuloma

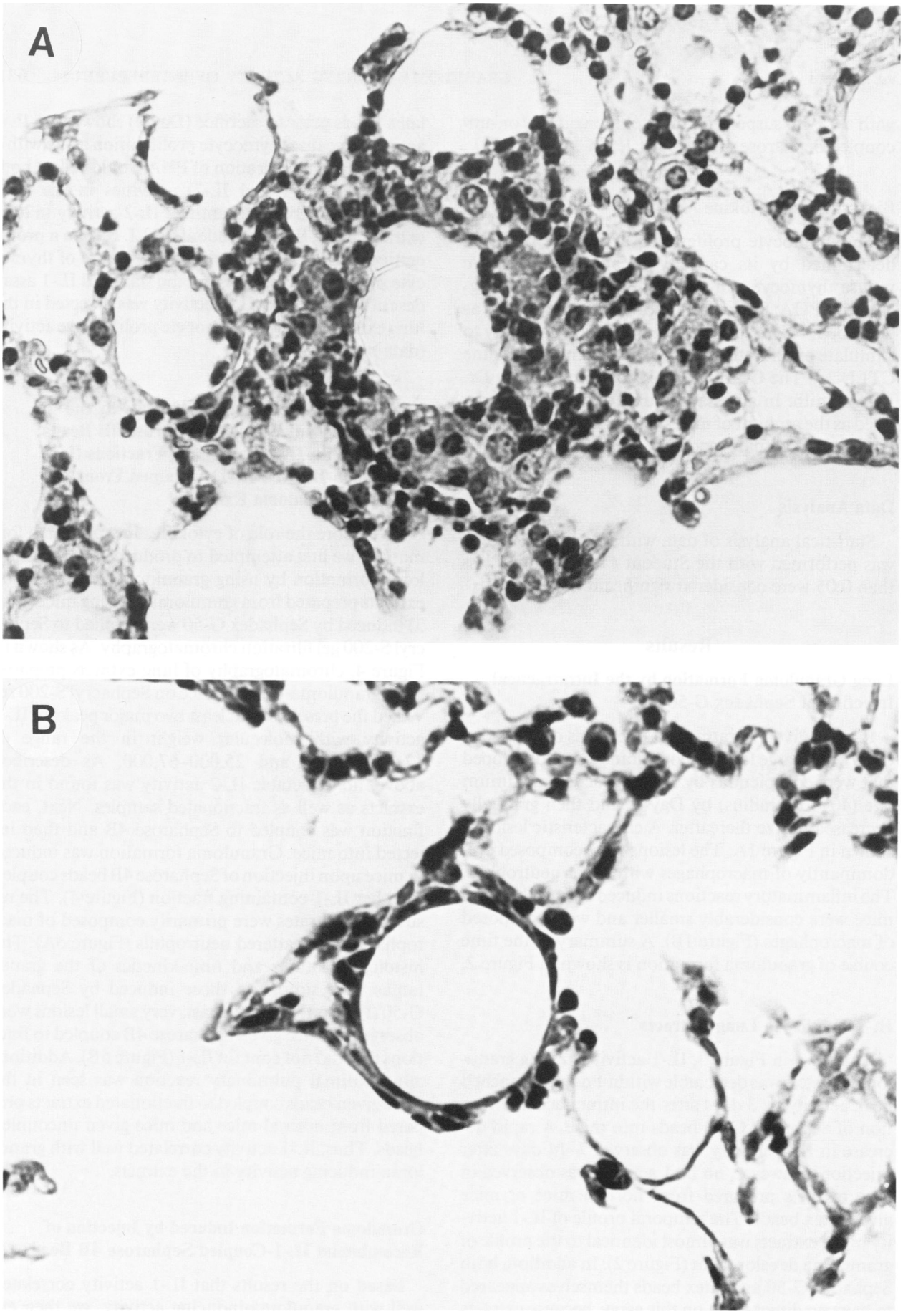

Figure 1—Pulmonary granulomas induced in BALB/c mice injected intratracheally with Sephadex G-50 or latex beads. **A**—Representative granuloma seen in a mouse sacrificed 3 days after intratracheal challenge with Sephadex G-50 beads. The inflammatory infiltrate surrounding the bead is composed predominantly of macrophages with fewer neutrophils. (H&E ×200) **B**—Inflammatory reaction in a mouse sacrificed 3 days after intratracheal injection with latex beads. Only a small number of macrophages surround the bead. (H&E ×200)

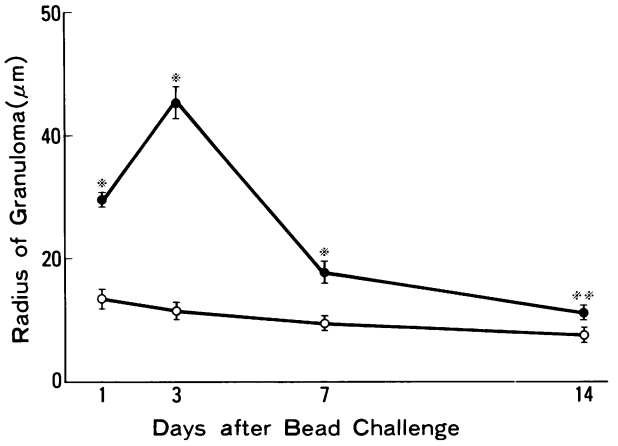

Figure 2—Kinetics of pulmonary granuloma formation induced in BALB/c mice sacrificed at various intervals after intratracheal injection of Sephadex G-50 (●) or latex beads (○). Ten randomly selected beads from a representative section of each lung were evaluated. Data represent the mean radius (microns) ± SEM of granulomatous inflammation from four independent experiments of three mice per each condition. Statistic difference relative to granuloma size in mice challenged with latex beads (*$P < 0.01$; **$P < 0.05$).

formation by using recombinant human IL-1. Because both human interleukins (IL-1 and IL-2) are active across species lines,[16,17] we used recombinant human IL-1 and IL-2 in this experiment. In mice injected intratracheally with recombinant IL-1-coupled Sepharose 4B beads large granulomas developed that reached maximum size (40–50 μ) by Day 3 (Figure 6A) and gradually decreased in size thereafter. The histologic features of recombinant IL-1-induced granulomas were similar to those induced by Sephadex G-50 (Figure 1A) or granuloma-derived IL-1-coupled Sepharose 4B beads (Figure 5A). Further-

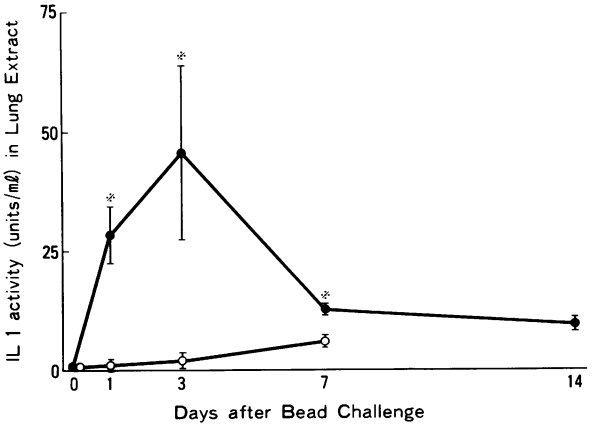

Figure 3—Demonstration of IL-1 activity in pulmonary granuloma extracts. Extracts prepared from mice given Sephadex G-50 (●) or latex (○) beads. Data represent the mean (units per milliliter) ± SEM in four separate experiments of 3 mice per each condition performed in triplicate. Statistic difference (*) relative to IL-1 activity in the extracts prepared from mice challenged with latex beads ($P < 0.05$).

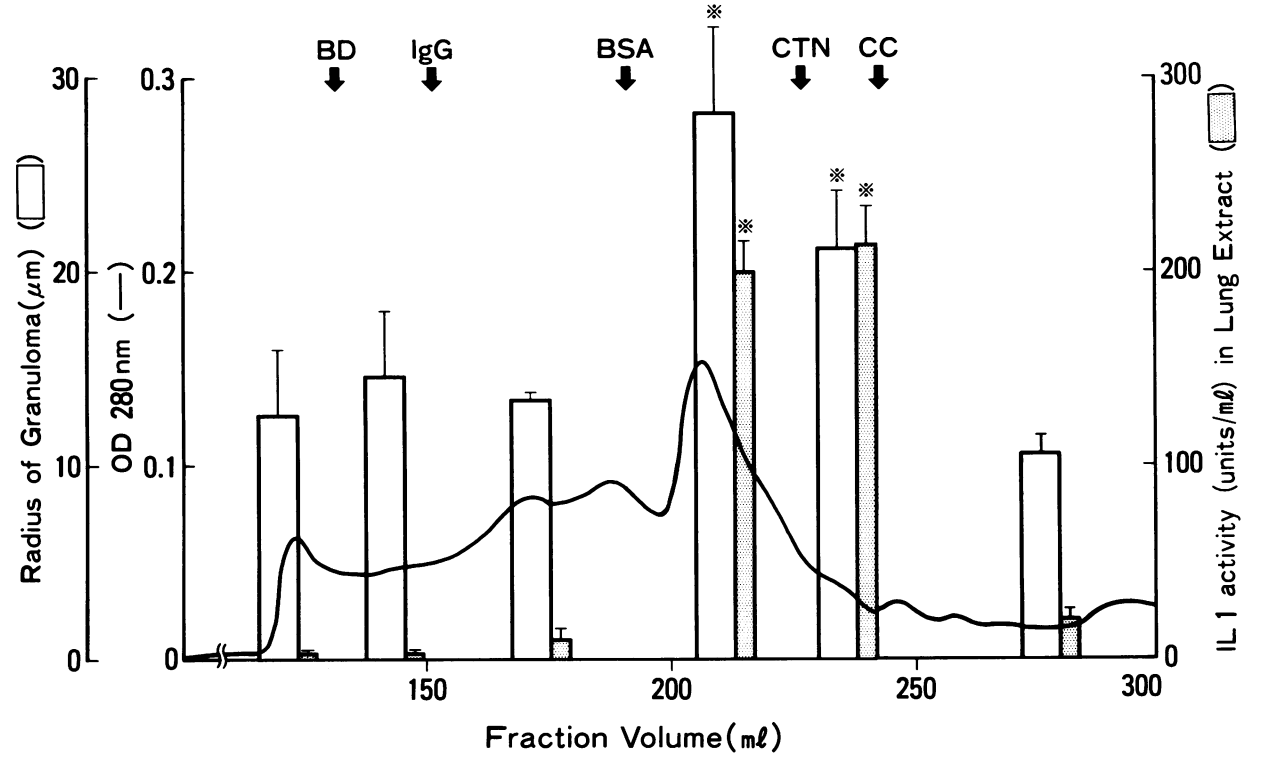

Figure 4—Fractionation of the extracts prepared from Sephadex G-50 induced lung granulomas (Day 3) on Sephacryl S-200. Each fraction was examined for IL-1 and granuloma-inducing activities. The granuloma-inducing activity in BALB/c mice was examined by intratracheal injection of Sepharose 4B beads coupled to each fraction and the mice sacrificed 3 days after the injection. These fractions did not contain any detectable IL-2 activity. Statistic difference (*) relative to controls ($P < 0.05$).

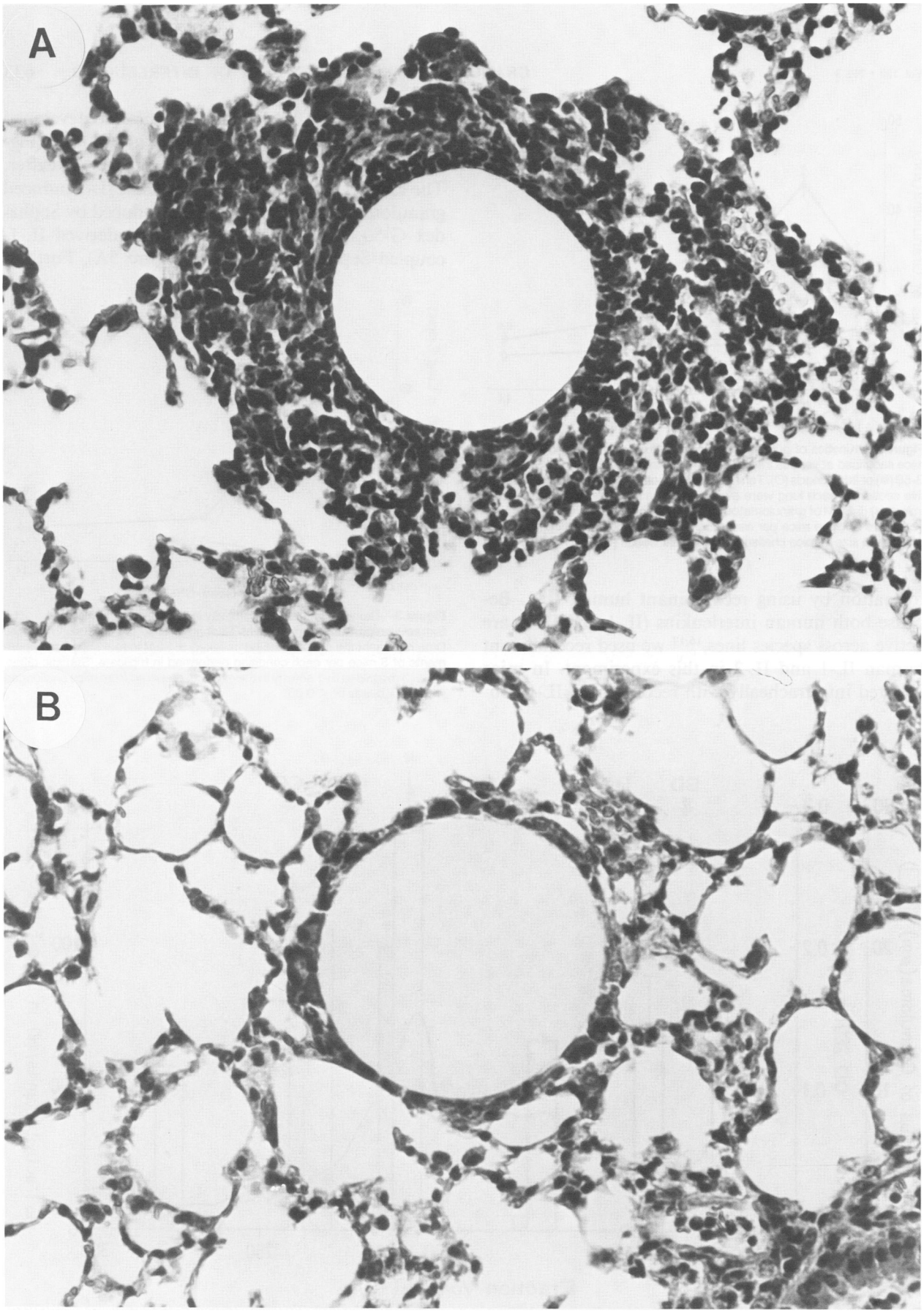

Figure 5—Histologic characteristics of the lung granulomas induced by intratracheal injection of Sepharose 4B beads coupled to extract fractions. The lung extracts were prepared from mice sacrificed 3 days after Sephadex G-50 challenge and were fractionated by Sephacryl S-200 gel filtration chromatography. **A**—Representative lesion seen in a mouse sacrificed 3 days after intratracheal injection of Separose 4B beads coupled to fractions containing IL-1 activity. A large granulomatous response is observed, and the cellular components of the lesion are mainly macrophages. (H&E ×200) **B**—Section from the lung of a mouse given 3 days previously Sepharose 4B coupled to fractions that did not contain IL-1 activity. The bead is surrounded by a very mild infiltrate of macrophages. (H&E ×200)

more, IL-1, but not IL-2, activity was detected in the extracts prepared from mice bearing granulomas induced by recombinant IL-1-coupled bead injection (data not shown). Again, the IL-1 activity in the lung extracts was associated with development of granulomas induced by IL-1-coupled beads. The dose of IL-1 activity that bound to beads was almost equivalent to the amount of its activity observed in lung extracts prepared from mice given Sephadex G-50. In contrast, the inflammatory reactions in mice induced by IL-2-coupled beads (Figure 6B) or plain beads (Figure 6C) were considerably smaller. No IL-1 activity was observed in the extracts prepared from mice given IL-2-coupled or plain beads (data not shown). The time course of granuloma formation induced by IL-1-coupled beads is shown in Figure 7.

Discussion

In the present study, we have demonstrated high levels of IL-1 activity in the extracts prepared from mice bearing pulmonary foreign-body granulomas induced by the intratracheal injection of dextran microparticles (Sephadex G-50). The extract fractions that showed IL-1 activity contained granulomatogenic factor(s). Additionally, intratracheal injection of Sepharose 4B beads coupled to recombinant IL-1, but not IL-2, was able to induce granulomas in mice. Again, the IL-1 activity in the lung extracts was correlated with development of granulomas induced by IL-1-coupled beads. These results strongly suggest that IL-1 possesses granuloma-inducing activity.

Granuloma formation is the expression of a series of complex inflammatory events. Evidence has accumulated in recent years that suggests that cytokines may play important roles in the initiation and maintenance of hypersensitivity granuloma formation.[3-7,9,18,19] In both hypersensitivity and foreign-body granuloma models, IL-1, but not IL-2, activity was detected in the granuloma extracts in association with activity/size of granulomas,[6,7,9] although there has been no direct evidence that supports the ability of IL-1 to induce granulomas. It has been reported that injection of dextran beads such as Sephadex G-50 or G-75 into mice induces foreign-body granuloma formation.[8,20] However, the exact mechanisms of foreign-body granuloma formation have not been clarified, to our knowledge. IL-1 activity in the extracts is a common feature in hypersensitivity,[6] tuberculosis,[7] and foreign-body granulomas, as shown in the present study (Figure 3), although migration inhibition factor was observed in hypersensitivity[6] and tuberculosis[7] but not in foreign-body granuloma model.[8] No detectable IL-2 activity was observed in the extracts prepared from three different models.[6,7,9] In the present paper, we have described that pulmonary granulomas can be induced in mice by the intratracheal injection of IL-1 coupled Sepharose 4B beads that serve as the nidi for the lesions. Taken together with the results of IL-1 activity in the extracts, these results suggest that IL-1 can initiate the induction of granulomas and may be involved in the fundamental mechanisms of both hypersensitivity and foreign-body granuloma formation. Because it has been reported that injection of beads coated with multiple lymphokine activities containing migration inhibition factor, chemotactic factors, and mitogenic factors is able to induce granulomas,[4,5] it should be elucidated whether other cytokines may be involved in the development of granulomas.

Although activated monocytes/macrophages produce a great number and variety of biologically active molecules,[21] cosiderable attention has been focused on IL-1 (or perhaps the IL-1 set of molecules) as an important mediator of immune and inflammatory responses.[16] The identity of IL-1 has been a controversial issue for many years. Biologic activity has been ascribed to molecular species derived from a plethora of cell types.[16] Polypeptides with molecular weights ranging from 2000 to 750,000 (17,000 is the predominant form) have been reported to have IL-1 biologic activity,[16,22] although it is generally accepted that smaller molecular weight species are proteolytic breakdown products, and the larger one represents an aggregated form of the molecules[23] and/or a complex of the lower molecular weight IL-1 with a serum component(s).[24] In this report we show that two major peaks of IL-1 as well as granulomatogenic activity with molecular weight in the range of 12,000–25,000 and 25,000–67,000 (Figure 4) may represent the molecular heterogeneity of IL-1.[23,24] Thus, IL-1 may exist free or in a complex form such as aggregation, glycosylation, and association with a component(s) of serum.[23,24] IL-1 has multiple effects on cells involved in inflammation, such as chemotaxis of polymorphonuclear cells[25,26] and macrophages.[16,26] In view of the predominant participation of macrophages in foreign-body granulomas (Figures 1, 5, and 6), it is reasonable that chemotactic activity[16,25,26] and inflammatory signals[16,27-29] of IL-1 may play an important role in the development and/or maintenance of granulomas. It should be noted that there are other possible sources of IL-1 activity beside macrophages (reviewed by Oppenheim et al[16]). Because granulomas were primarily composed of macrophages and recombinant IL-1β used in these experiments was originated

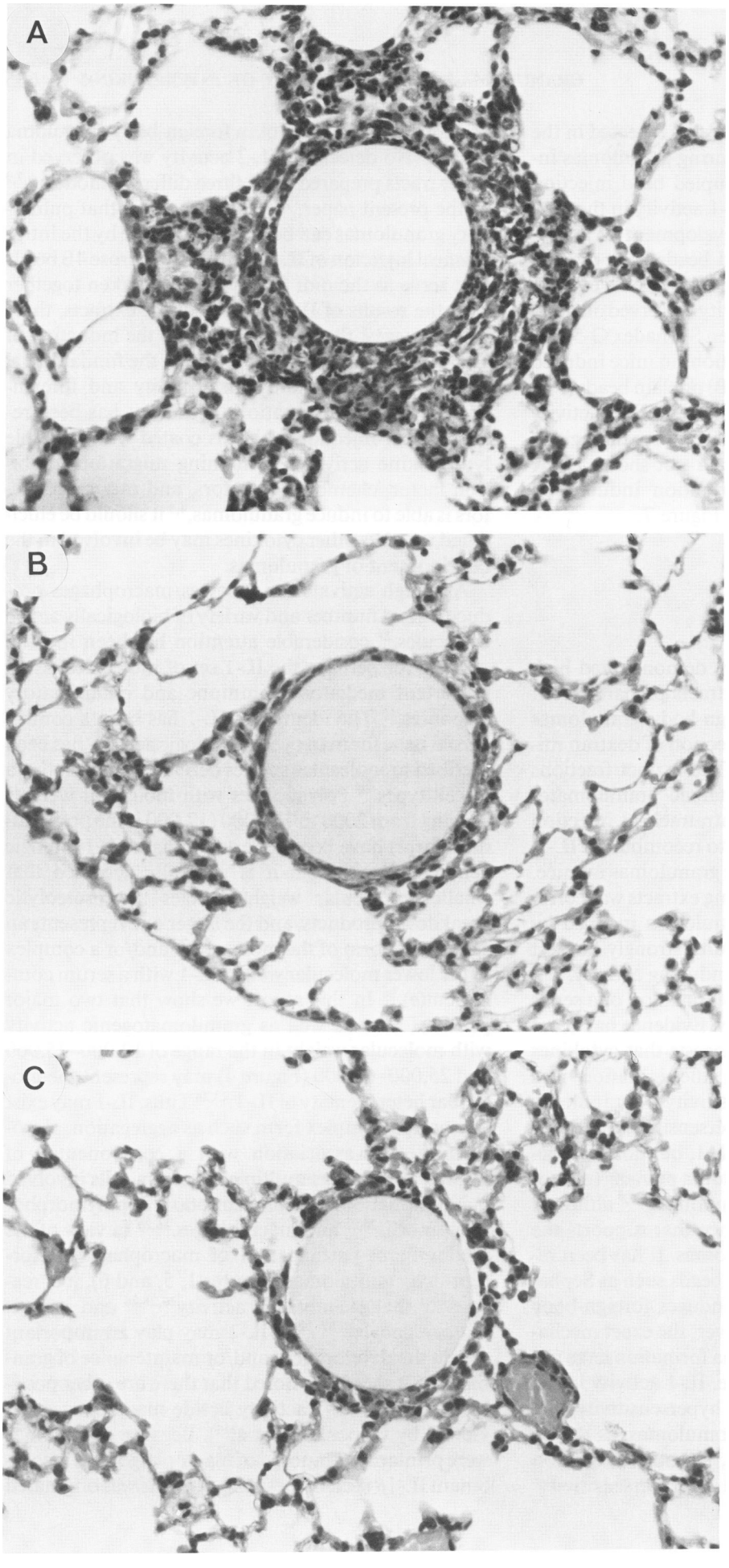

Figure 6—Granuloma formation by intratracheal injection of Sepharose 4B beads coupled to recombinant IL-1. **A**—Representative granuloma seen in a mouse sacrificed 3 days after intratracheal injection with recombinant IL-1-coupled Sepharose 4B beads. A large granuloma is observed, and the cellular components are mainly macrophages. (H&E ×200) **B**—Lung section of a mouse given 3 days previously recombinant IL-2-coupled beads. Only a small number of macrophages surround the bead. (H&E ×200) **C**—Inflammatory reaction in a mouse sacrificed 3 days after injection with uncoupled Sepharose 4B beads. The bead is surrounded by a very mild infiltrate of macrophages. (H&E ×200)

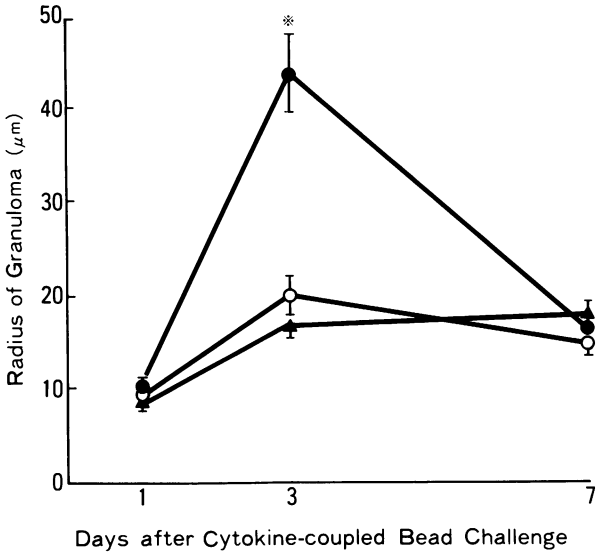

Figure 7—Kinetics of granuloma formation in BALB/c mice given cytokine-coupled Sepharose 4B beads. Mice were challenged intratracheally with recombinant IL-1-coupled (●), recombinant IL-2-coupled (▲), or plain beads (○). The data represent the mean radius (microns) ± SEM of the radius of granulomatous inflammation compiled from three separate experiments of 3 mice per each condition. Significant difference (*) when compared with IL-2-coupled or uncoupled Sepharose 4B bead injection ($P < 0.01$).

from human peripheral blood monocytes,[10] it is most likely that the thymocyte-activating factor in the extracts is due to macrophage-derived IL-1.

The results described in this paper provide evidence for the ability of IL-1 to induce granuloma formation *in vivo*. The data reported here and from our previous studies[6-9] suggest that this model of granulomatous inflammation may be a useful tool for investigating not only the mechanisms of granuloma formation but also the *in vivo* role of cytokines.

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